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Full paper

Effects of 071031B, a novel serotonin and norepinephrine reuptake inhibitor, on monoamine system in mice and rats

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ABSTRACT

Our previous study indicated that 071031B, a novel potential serotonin and norepinephrine reuptake inhibitor, showed robust antidepressant activity in multiple depression models, and could simultaneously inhibit 5-HT and NE reuptake in vitro. The present study was to evaluate the effects of 071031B on monoamine system in vivo, by using pharmacological models, including 5-HTP induced head-twitch test, yohimbine toxicity potentiation test, and reserpine induced hypothermia test, and determining monoamine transmitter levels in reserpine induced monoamine depletion model or chronic unpredictable stress (CUS) model. Results in pharmacological models indicated that acute administration of 071031B at 5–20 mg/kg significantly enhanced 5-HTP induced head-twitch behavior, potentiated yohimbine induced lethal rate, and reversed reserpine induced hypothermia. Further monoamine assays demonstrated that acute or chronic administration of 071031B at 10 or 20 mg/kg increased 5-HT and/or NE levels in various brain regions in reserpine or CUS induced monoamine depletion models, respectively, without effect on DA and its metabolites. Our results revealed that 071031B produces potent inhibition of 5-HT and NE reuptake in vivo.

1. Introduction

Depression is a complicated psychiatric disorder with various types of symptoms, which suggest that depression is induced by complicated interaction within multiple factors and systems. Within various etiology hypotheses, monoamine hypothesis supports that low levels of monoamines, especially serotonin (5-HT) and norepinephrine (NE), contribute to the occurrence of depression (1). Drug induced enforcement on monoamine system is beneficial in depression therapy, and can be realized by three strategies, inhibiting monoamine reuptake, e.g., fluoxetine, reboxetine, or duloxetine targeting at monoamine transporters, or inhibiting monoamine metabolism, e.g., moclobemide targeting at monoamine oxidase A, or increasing monoamine release indirectly, e.g., agomelatine, marketed as the first antidepressant beyond monoaminergic targets, exerts antidepressant effects partially depending on its antagonistic activity at 5-HT_{2C} receptors and subsequent outflows of NE and dopamine (DA) (2). 5-HT and NE are the most crucial transmitters in depression etiology and therapy, albeit their effects were not the same. 5-HT enhancement was closely related with emotion improvement, while NE was reported to show positive effect on motivation or anhedonia (3). These may partially support that dual reuptake inhibitors show better efficacy than the single ones (4).

Our previous studies indicated that a novel serotonin and norepinephrine reuptake inhibitor (SNRIs) 071031B ((±)-3-(benzo[*d*][1,3]dioxol-4-yloxy)-*N*-methyl-3-(thiophen-2-yl) propan-1-amine, Fig. 1) showed robust antidepressant activity in multiple depression models, i.e., tail suspension test in mice, forced swimming test in mice and rats, and chronic unpredictable stress model in rats (5, 6). Target research indicated that 071031B could bind to 5-HT transporter (SERT) and NE transporter (NET) simultaneously and then inhibit these two transmitters' reuptake in vitro, which

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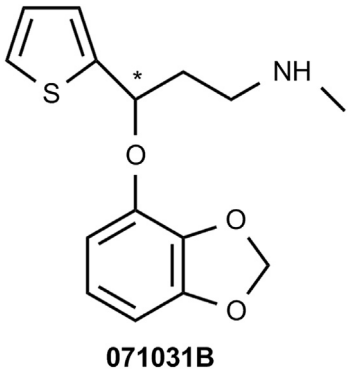

Fig. 1. Chemical structure of 071031B.

may be the mechanisms underlying its antidepressant efficacy (5). However, it should be noted that in vitro data from binding or uptake may not mimic and then represent true process in vivo. We all know that neuron discharge and neurotransmitter release is mediated by auto receptors, i.e., increase of 5-HT or NE would activate 5-HT_{1A} or α_2 auto receptors and this activation would in turn suppress neuron discharge and then reduce transmitter release, and the further reciprocal interactions between 5-HT and NE systems make the actual process of neurotransmitter release more complicated (7). What are the final results following these interactions? Accordingly, in vivo process is more complicated that can not be speculated by in vitro data. The present study was to systematically evaluate the effects of 071031B on monoamine system in vivo, by using pharmacological models, including 5-HTP induced head-twitch test, yohimbine toxicity potentiation test, and reserpine induced hypothermia test, and determining monoamine transmitter levels in reserpine induced monoamine depletion model or chronic unpredictable stress model.

2. Materials and methods

2.1. Animals

Unless specified below, male ICR mice weighing 18–20 g and male Sprague–Dawley rats weighing 180–200 g were used (Beijing Vital River Laboratory Animal Technology Company, Beijing, China). Animals were group housed under standard condition as previously described (5). Experiments were approved by the local Animal Welfare Committee for Animal Experiments at Beijing Institute of Pharmacology and Toxicology, and were performed in compliance with Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

2.2. Materials

Duloxetine hydrochloride was purchased from Beijing Furenkang Biopharmaceutical Corporation, Ltd (Beijing, China). 071031B oxalate was synthesized at Beijing Institute of Pharmacology and Toxicology. Fluoxetine, 5-hydroxy-L-tryptophan (5-HTP), yohimbine hydrochloride, reserpine hydrochloride, 5-HT, DA, NE, 5-hydroxyindole-3-acetic acid (5-HIAA), homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were purchased from Sigma Aldrich (St. Louis, MO, USA). Vehicle or drugs were dissolved in distilled water and administered orally (p.o.), intraperitoneally (i.p.), or subcutaneously (s.c.) in a volume of 10 ml/kg for mice and 2 ml/kg for rats.

2.3. 5-HTP induced head-twitch test in mice

The test was performed as previously described (8, 9). Sixty minutes after acute p.o. administration of vehicle, duloxetine, or various doses of 071031B, 5-HTP (120 mg/kg) were injected i.p., and then the number of head-twitches (rapid movements of the head with little or no involvement of the trunk) was counted immediately for 20 min.

2.4. Yohimbine toxicity potentiation test in mice

The procedure was performed as previously described (9, 10). Sixty minutes after acute p.o. administration of vehicle, duloxetine, or 071031B at various doses, yohimbine (30 mg/kg) was injected s.c., and then the number of death was recorded in the following 24 h.

2.5. Reserpine induced hypothermia test in rats

The test was performed as previously described with minor modifications (11). Reserpine (5 mg/kg) was injected i.p. 17 h before drug treatment. Seventeen hours later, rectal temperature was recorded as basal temperature (t0, °C), and then, vehicle, duloxetine, and 071031B at various doses were administered i.p. immediately. Sixty minutes after drug treatment, rectal temperature was measured again (t1, °C). The difference ($\triangle$T, °C) between t0 and t1 was calculated. After temperature assays, various regions of rat brain were isolated to assess the effects of reserpine and 071031B on levels of monoamines and their metabolites using high performance liquid chromatography with electrochemical detection (HPLC-ECD).

2.6. Chronic unpredictable stress procedure

The chronic unpredictable stress procedure was developed as previously described (5). Rats were divided into six groups: Control (non-stress), Stress-Vehicle (distilled water), Stress-Fluoxetine (10 mg/kg), Stress-Duloxetine (10 mg/kg), Stress-071031B (5 or 10 mg/kg). Drugs were administered orally once daily for 4 weeks. Except for control group, rats were subjected to a variety of stressors described in Fig. 2 and Table 1 and 4 weeks later, the open-field test, the sucrose preference test, and the novelty-suppressed feeding test were performed. The behavioral results of 071031B in CUS model have been reported in our previous research (5), and brains from the same rats were used to assess the effects of stress and 071031B on levels of monoamines and their metabolites using HPLC-ECD.

2.7. Monoamine assays by HPLC-ECD

Measurements of monoamine transmitters (5-HT, NE, DA) and their metabolites (5-HIAA, DOPAC, HVA) in various brain regions were performed using HPLC-ECD as previously described (9). Briefly, tissue samples were homogenized in 0.4 N perchloric acid with 0.5 mM Na_2-EDTA and 0.01% L-cysteine (5 or 10 μl/mg of tissue), and were centrifuged for 30 min at speed of 12,000 rpm at 4 °C. The supernatant were collected and analyzed. The HPLC system, the mobile phase consistence and other specific details were referred to the protocol established by our team (9). Sample levels of monoamines and related metabolites were quantified according to standard curve, taking area under curve (AUC) as an index.

2.8. Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Prism 5.0, version 2.0; GraphPad Software Inc., San Diego,

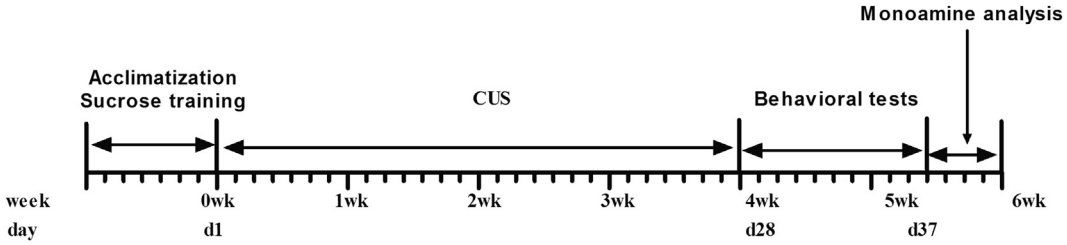

Fig. 2. Chronic unpredictable stress (CUS) regime.

Table 1
Chronic unpredictable stress regime.

Day	Week			
	Week 1	Week 2	Week 3	Week 4
Sunday	White noise: 1 h Overnight stroboscope: 12 h	Cage tilt: 24 h	Cage tilt: 24 h	Cage tilt: 24 h
Monday	Food deprivation: 24 h	Shock: 30 min	Shock: 30 min	Forced swimming: 5 min
Tuesday	Restraint: 1 h Overnight illumination: 12 h	White noise: 2 h Overnight illumination: 12 h	Water deprivation: 24 h	Soiled cage: 24 h
Wednesday	Tail pinch: 1 min	Restraint: 2 h Overnight stroboscope: 12 h	Tail pinch: 1 min	Shock: 30 min
Thursday	Soiled cage: 24 h	Soiled cage: 24 h	Soiled cage: 24 h	Tail pinch: 1 min
Friday	Forced swimming: 5 min	Forced swimming: 5 min	Restraint: 2 h Overnight stroboscope: 12 h	Food deprivation: 24 h
Saturday	Water deprivation: 24 h	Food deprivation: 24 h	Water deprivation: 24 h	Cage tilt: 24 h

CA). Unless otherwise specified, data were analyzed using Student's t-test or one-way analysis of variance (ANOVA) followed by Dunnett's test. For data that exhibiting unequal variances, Mann–Whitney U test or Kruskal–Walis test followed by Dunn's Multiple Comparison Test was applied. Data form yohimbine toxicity potentiation test in mice were analyzed using Fisher's exact test.

3. Results

3.1. Effects of 071031B on 5-HTP induced head-twitch behavior

Fig. 3 shows that co-administration of 071031B (5, 10, or 20 mg/kg, p.o.) and 5-HTP elicited a pronounced head-twitch behavior in mice in a dose-dependent manner (one-way ANOVA, $F4,45 = 4.281$; $p = 0.0051$). Further post-hoc analysis revealed that 071031B (p.o.) at the doses of 10 and 20 mg/kg significantly increased the number of head-twitches (Dunnett's test, as compared with vehicle, $p < 0.05$ for 10 mg/kg, $p < 0.01$ for 20 mg/kg, respectively). Similar effect was produced by duloxetine at 20 mg/kg (Dunnett's test, $p < 0.05$).

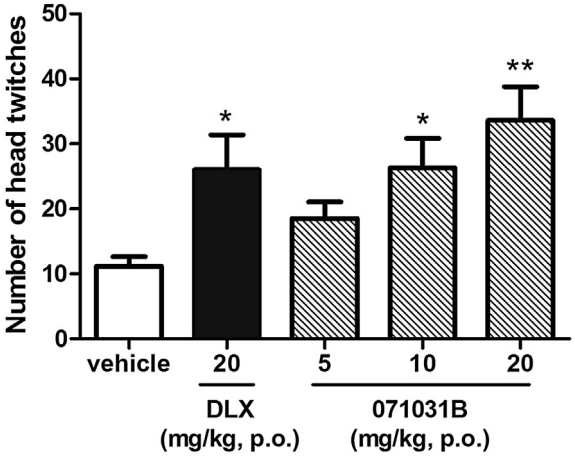

Fig. 3. Effects of 071031B (5–20 mg/kg) or duloxetine (20 mg/kg) on 5-HTP induced head-twitch behavior in mice. Vehicle (VEH), duloxetine (DLX), or 071031B was administered p.o. 60 min prior to 5-HTP (120 mg/kg, i.p.) injection. Data are presented as means ± S.E.M. ($n = 10$/group). $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* VEH.

3.2. Effects of 071031B on yohimbine induced lethal toxicity

As shown in Table 2, yohimbine injection only induced 10% mortality rate, while combined administration of 071031B at doses of 5–20 mg/kg significantly increased the mortality rates to 100%, 90% or 100%, respectively (Fisher's exact test, as compared with vehicle, $p = 0.0001$ for 5 mg/kg, $p = 0.0011$ for 10 mg/kg, $p = 0.0001$ for 20 mg/kg, respectively). Similar effect was produced by duloxetine at 20 mg/kg ($p = 0.0001$).

3.3. Effects of 071031B on reserpine induced hypothermia response

Intraperitoneal injection of reserpine at 5 mg/kg induced obviously hypothermia response. As shown in Fig. 4, 071031B at 5–20 mg/kg significantly reduced reserpine induced hypothermia, as evidenced by the decreased △T/(°C) values (one-way ANOVA, $F4,43 = 7.600$, $p = 0.0001$; Dunnett's test was chose as post-hoc test, as compared with VEH, $p < 0.001$ for 5 mg/kg, $p < 0.001$ for

Table 2
Effects of 071031B on the mortality rate in the yohimbine toxicity potentiation test in mice.

Group	Dose/(mg/kg)	Number of death in 24 h	Mortality rate in 24 h/(%)
Vehicle	–	1	10
DLX	20	10	100***
071031B	5	10	100***
	10	9	90***
	20	10	100***

$^{***}P < 0.001$ versus vehicle, Fisher's exact test. $n = 10$.

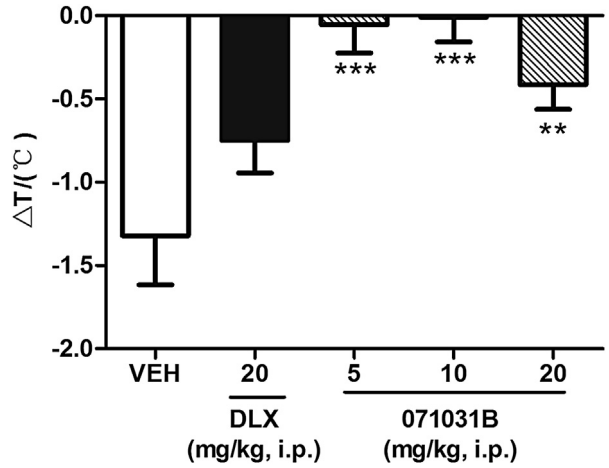

Fig. 4. Effects of 071031B (5–20 mg/kg) or duloxetine (20 mg/kg) on reserpine-induced hypothermia in rats. Reserpine (5 mg/kg) was injected i.p. 17 h before vehicle (VEH), duloxetine (DLX), or 071031B treatment, and rectal temperature was measured sixty minutes after drug treatment. Data are presented as means ± S.E.M. ($n = 10$/group). $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* VEH.

10 mg/kg, $p < 0.01$ for 20 mg/kg, respectively). However, duloxetine at 20 mg/kg administered 1 h before test did not produce antagonizing effect on body temperature in resperpinized rats (Dunnett's test, $p > 0.05$).

3.4. Effects of 071031B on reserpine induced monoamine changes

Table 3 illustrates the effects of 071031B or duloxetine on the levels of monoamine and metabolites in prefrontal cortex, hippocampus, hypothalamus and striatum in reserpinized rats. As compared with vehicle (reserpine (−)), pretreatment of reserpine at 5 mg/kg significantly decreased levels of 5-HT and NE, and increased levels of 5-HIAA/5-HT, in prefrontal cortex (as for 5-HT, Mann Whitney test, $U = 0.0000$, $p = 0.0025$; as for NE, Student's t-test, $t = 9.444$, $p < 0.0001$; as for 5-HIAA/5-HT, Mann Whitney test, $U = 0.0000$, $p < 0.01$), hippocampus (as for 5-HT, Student's t-test, $t = 4.713$, $p = 0.0006$; as for NE, Student's t-test, $t = 4.678$, $p = 0.0005$; as for 5-HIAA/5-HT, Mann Whitney test, $U = 0.0000$, $p = 0.0022$) and hypothalamus (as for 5-HT, Mann Whitney test, $U = 0.0000$, $p = 0.0016$; as for NE, Mann Whitney test, $U = 0.0000$, $p = 0.0025$; as for 5-HIAA/5-HT, Mann Whitney test, $U = 0.0000$, $p < 0.01$); reserpine also decreased DA (Student's t-test, $t = 23.51$, $p < 0.0001$) and increased DOPAC levels (Student's t-test, $t = 13.79$, $p < 0.0001$) in striatum.

In prefrontal cortex, 071031B showed increasing effects on levels of 5-HT (Kruskal–Walis test, Kruskal–Walis statistic 4,36 = 21.13, $p = 0.0003$) or NE (one-way ANOVA, $F4,38 = 2.264$, $p = 0.0802$) and decreasing effects on those of 5-HIAA/5-HT (Kruskal–Walis test, Kruskal–Walis statistic 4,36 = 25.32, $p < 0.0001$), and post-hoc analysis revealed that, as compared with reserpine group, it was significant at the dose of 20 mg/kg for 5-HT (Dunn's Multiple Comparison Test, $p < 0.001$), 10 mg/kg for NE (Dunnett's test, $p < 0.05$) and 20 mg/kg for 5-HIAA/5-HT (Dunn's Multiple Comparison Test, $p < 0.001$). Duloxetine at 20 mg/kg showed no effect on 5-HT ($p > 0.05$), increasing effect on NE ($p < 0.05$), and decreasing effect on 5-HIAA/5-HT ($p < 0.05$) levels.

In hippocampus, 071031B or duloxetine showed no effect on levels of 5-HT (Kruskal–Walis test, Kruskal–Walis statistic 4,36 = 4.555, $p = 0.336$) and NE (Kruskal–Walis test, Kruskal–Walis statistic 4,40 = 0.7670, $p = 0.9428$), while reserpine induced 5-HIAA/5-HT enhancement was antagonized by 071031B at 5–20 mg/kg and duloxetine at 20 mg/kg (one-way ANOVA followed, $F4,33 = 2.650$, $p = 0.0506$; Dunnett's test, $p < 0.05$ for 071031B at all doses and duloxetine at 20 mg/kg, respectively).

In hypothalamus, 071031B showed increasing effects on levels of 5-HT (Kruskal–Walis test, Kruskal–Walis statistic 4,36 = 23.71, $p < 0.0001$) and NE (Kruskal–Walis test, Kruskal–Walis statistic 4,36 = 14.69, $p = 0.0054$) and decreasing effects on those of 5-HIAA/5-HT (Kruskal–Walis test, Kruskal–Walis statistic 4,35 = 24.27, $p < 0.0001$). Post-hoc analysis revealed that, as compared with reserpine group, 071031B at 20 mg/kg significantly enhanced both 5-HT and NE levels and decreased 5-HIAA/5-HT values (Dunn's Multiple Comparison Test, $p < 0.01$, $p < 0.05$, $p < 0.01$, respectively), 071031B at 10 mg/kg decreased 5-HIAA/5-HT values (Dunn's Multiple Comparison Test, $p < 0.05$), and 071031B at 5 mg/kg showed no effect on these three measurements. Duloxetine at 20 mg/kg showed similar increasing effects on 5-HT and NE levels and decreasing effects on 5-HIAA/5-HT values

Table 3
Effects of 071031B on levels of monoamines and related metabolites (ng/mg) in prefrontal cortex, hippocampus, striatum and hypothalamus in reserpinized rats.

Groups	Reserpine (−)	Reserpine + VEH	Reserpine + DLX	Reserpine + 071031B		
			20 (mg/kg)	5(mg/kg)	10 (mg/kg)	20 (mg/kg)
Prefrontal cotex						
5-HT	0.475 ± 0.111	0.021 ± 0.007**	0.070 ± 0.077	0.035 ± 0.035	0.058 ± 0.088	0.141 ± 0.047$^{\#\#\#}$
5-HIAA	0.395 ± 0.071	0.628 ± 0.059***	0.402 ± 0.150$^{\#\#}$	0.497 ± 0.124	0.442 ± 0.136$^{\#}$	0.437 ± 0.128$^{\#}$
5-HIAA/5-HT	0.856 ± 0.166	33.630 ± 12.180**	7.537 ± 4.729$^{\#}$	22.790 ± 12.35	17.790 ± 16.08	3.300 ± 1.149$^{\#\#\#}$
NE	0.570 ± 0.085	0.157 ± 0.060***	0.297 ± 0.081$^{\#}$	0.245 ± 0.129	0.296 ± 0.122$^{\#}$	0.241 ± 0.070
Hippocampus						
5-HT	0.492 ± 0.171	0.088 ± 0.138***	0.065 ± 0.053	0.033 ± 0.025	0.077 ± 0.123	0.110 ± 0.120
5-HIAA	0.659 ± 0.165	1.012 ± 0.268*	0.558 ± 0.122$^{\#\#\#}$	0.860 ± 0.290	0.742 ± 0.157$^{\#}$	0.685 ± 0.121$^{\#}$
5-HIAA/5-HT	1.430 ± 0.397	342.800 ± 558.100**	15.240 ± 16.010$^{\#}$	40.820 ± 28.100$^{\#}$	31.760 ± 21.700$^{\#}$	19.660 ± 17.910$^{\#}$
NE	0.596 ± 0.097	0.257 ± 0.155***	0.219 ± 0.105	0.221 ± 0.088	0.299 ± 0.182	0.210 ± 0.044
Hypothalamus						
5-HT	0.536 ± 0.091	0.034 ± 0.012**	0.162 ± 0.052$^{\#\#\#}$	0.077 ± 0.036	0.072 ± 0.035	0.131 ± 0.069$^{\#\#}$
5-HIAA	0.536 ± 0.094	0.812 ± 0.258*	0.634 ± 0.300	0.746 ± 0.282	0.448 ± 0.091$^{\#\#}$	0.642 ± 0.216
5-HIAA/5-HT	1.020 ± 0.239	27.510 ± 7.004**	4.359 ± 2.454$^{\#\#\#}$	13.280 ± 9.599	7.016 ± 4.391$^{\#}$	5.867 ± 2.926$^{\#\#}$
NE	10.783 ± 2.091	0.161 ± 0.061**	0.427 ± 0.226$^{\#\#}$	0.287 ± 0.101	0.246 ± 0.057	0.437 ± 0.290$^{\#}$
Striatum						
DA	7.400 ± 0.660	0.284 ± 0.490***	0.438 ± 0.160	0.303 ± 0.136	0.268 ± 0.143	0.355 ± 0.330
DOPAC	2.702 ± 0.335	0.837 ± 0.211***	1.022 ± 0.289	0.908 ± 0.177	0.844 ± 0.220	0.798 ± 0.158
HVA	0.647 ± 0.114	0.484 ± 0.202	0.663 ± 0.343	0.720 ± 0.464	0.566 ± 0.238	0.544 ± 0.210

Values are expressed as means ± S.D. ($n = 5–10$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus Reserpine (−), Student's t-test or Mann–Whitney U test; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus Reserpine + VEH, ANOVA followed by Dunnett's test or Kruskal–Walis test followed by Dunn's Multiple Comparison Test.

(Dunn's Multiple Comparison Test, $p < 0.01$, $p < 0.05$, $p < 0.001$, respectively).

In striatum, both 071031B and duloxetine showed no effect on levels of DA (one-way ANOVA, $F4,34 = 2.264$, $p = 0.6987$), DOPAC (one-way ANOVA, $F4,42 = 1.449$, $p = 0.2350$), or HVA (one-way ANOVA, $F4,42 = 0.9171$, $p = 0.4630$).

3.5. Effects of 071031B on CUS induced monoamine changes

Table 4 illustrates the effects of 071031B or duloxetine on levels of monoamine and metabolites in prefrontal cortex and striatum in CUS rats.

In prefrontal cortex, as compared with vehicle (stress (−)), four-week stress induced pronounced decreasing in levels of NE (Student's t-test, $t = 2.761$, $p = 0.0162$), while levels of 5-HT (Student's t-test, $t = 0.4675$, $p = 0.6473$) or 5-HIAA (Student's t-test, $t = 0.9219$, $p = 0.3722$) showed no change. As compared with stress group, 071031B, fluoxetine, or duloxetine showed increasing effects on levels of NE (one-way ANOVA, $F4,30 = 3.438$, $p = 0.0209$) and 5-HT (one-way ANOVA, $F4,30 = 0.2968$, $p = 0.0277$), decreasing effects on those of 5-HIAA (one-way ANOVA, $F4,30 = 10.11$, $p < 0.0001$). Post-hoc analysis revealed that 071031B at 10 mg/kg, duloxetine at 10 mg/kg, or fluoxetine at 10 mg/kg, could significantly increase levels of NE in CUS rats (Dunnett's test, $p < 0.05$, $p < 0.05$, $p < 0.05$, respectively), without effect on that 5-HT (Dunnett's test, $p > 0.05$, $p > 0.05$, $p > 0.05$, respectively). Fluoxetine at 10 mg/kg also decreased 5-HIAA levels (Dunnett's test, $p < 0.001$) in CUS rats.

In striatum, as compared with vehicle (stress (−)), four-week stress showed no effect on levels of DA, DOPAC, or HVA (Student's t-test; for DA, $t = 0.6652$, $p = 0.5185$; for DOPAC, $t = 0.4723$, $p = 0.6452$; for HVA, $t = 0.880$, $p = 0.3948$). As compared with stress group, 071031B, fluoxetine, or duloxetine showed no effect on levels of DA, DOPAC, or HVA (one-way ANOVA; for DA, $F4,28 = 0.6052$, $p = 0.6622$; for DOPAC, $F4,28 = 0.3972$, $p = 0.8089$; for HVA, $F4,28 = 2.035$, $p = 0.1166$).

4. Discussion

The inhibition of reuptake of 5-HT/NE in vitro, and the enhancing of serotonergic and noradrenergic transmission in vivo are characteristics of SNRIs. Our previous study have demonstrated that 071031B is an inhibitor of both 5-HT and NE transporters, and inhibits these two transmitters reuptake in brain in vitro (5). The present study demonstrated that 071031B produced serotonergic and noradrenergic neuronal activation in vivo, for it could significantly increase 5-HTP induced head −twitch number, potentiate toxicity caused by yohimbine and reverse hypothermia induced by reserpine, and most importantly, 071031B could block the depletion of 5-HT and/or NE induced by reserpine or chronic stress.

Additional injection of 5-HTP, the precursor of 5-HT, increases 5-HT levels abruptly in brain, and antidepressants, that exert potential activating effect on serotonergic system, will further enhance 5-HT concentration and then induce rapid head-twitch movement by activating 5-HT_{2A} receptor (8). Our results indicated that combination of 071031B and 5-HTP induced obvious head-twitch behavior, indicating that 071031B enhanced serotonergic system function in vivo. Duloxetine at 20 mg/kg showed similar effect, which was in accordance with the findings of Katoh et al. (12).

Yohimbine at ultra dose, by antagonizing alpha2 receptor and then increasing central NE concentration sharply, will lead to the death of mice (10). Co-administration of antidepressants, who have potential activating role in central noradrenergic system, will potentiate toxicity of yohimbine. In the current study, we demonstrated that co-administration of 071031B at 5–20 mg/kg and yohimbine caused almost 100% mortality, suggesting 071031B activated central noradrenergic system in vivo.

Reserpine could induce hypothermia action in animals or human by depleting NE levels, and antidepressants that have potential activating role in central noradrenergic system will inhibit or reverse this response. The present study demonstrated that 071031B at 5–20 mg/kg effectively reversed reserpine induced hypothermia, suggesting 071031B activated central noradrenergic system in vivo, yet this action showed a biphasic U-shape curve, i.e., the inhibition effect of 071031B at higher dose (20 mg/kg) was weaker than that at lower doses (5 or 10 mg/kg). In fact, Katoh et al (12) found that duloxetine showed similar characteristics, and their explanation was that high doses (25 and 50 mg/kg) of duloxetine may affect body temperature directly. Furthermore, in our study, duloxetine at 20 mg/kg could not significantly reduce reserpine induced hypothermia, which seemed to be inconsistent with previous study by Katoh et al. However, it should be noted that, studies from Katoh's lab and ours' used totally different experiment procedures. Firstly, rats were used in our study while mice were used in Katoh's study; secondly, the dose of reserpine was 5 mg/kg and 1 mg/kg in our and Katoh's study, respectively; then, in our study, we first injected reserpine and 17 h later, we gave duloxetine, then 1 h later, we tested mice's body temperature; whereas in Katoh's study, reserpine was injected 1hr after the administration of duloxetine, and the temperature was measured at various time points, yet not more than 5hr. Moreover, pharmacokinetic properties may also be a crucial factor contributing to duloxetine's invalid effect in the current study, for we only tested body temperature at one time point, i.e., 18 h. Results from pharmacological models demonstrated that 071031B could activate both 5-HT and NE system in vivo. Subsequently, we want to ensure whether this activation was realized by increasing 5-HT and NE transmitter's concentration.

Table 4
Effects of 071031B on levels of monoamines and related metabolites (ng/mg) in prefrontal cortex and striatum in CUS rats.

Groups	Stress (−)	Stress + VEH	Stress + FLX	Stress + DLX	Stress + 071031B	
				10 (mg/kg)	5 (mg/kg)	10 (mg/kg)
Prefrontal cotex						
5-HT	0.956 ± 0.128	0.929 ± 0.108	0.846 ± 0.085	1.039 ± 0.162	0.988 ± 0.131	1.041 ± 0.116
5-HIAA	0.883 ± 0.162	0.817 ± 0.121	0.541 ± 0.153$^{\#\#\#}$	0.867 ± 0.099	0.944 ± 0.126	0.758 ± 0.129
5-HIAA/5-HT	0.948 ± 0.253	0.901 ± 0.225	0.643 ± 0.186$^{\#}$	0.842 ± 0.099	0.958 ± 0.086	0.732 ± 0.130
NE	0.808 ± 0.119	0.682 ± 0.062*	0.818 ± 0.187$^{\#}$	0.863 ± 0.118$^{\#}$	0.823 ± 0.122	0.878 ± 0.110$^{\#}$
Striatum						
DA	17.206 ± 5.292	19.361 ± 6.458	18.563 ± 6.214	21.225 ± 3.124	21.976 ± 4.840	22.124 ± 3.753
DOPAC	3.799 ± 1.666	4.287 ± 2.076	3.665 ± 0.889	3.627 ± 1.504	4.790 ± 2.094	4.153 ± 2.650
HVA	1.008 ± 0.199	0.906 ± 0.244	0.816 ± 0.226	1.015 ± 0.181	1.129 ± 0.267	0.933 ± 0.139

Values are expressed as means ± S.D. ($n = 5–8$). $^{*}P < 0.05$ versus Stress (−),Student's t-test; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ versus Stress + VEH, ANOVA followed by Dunnett's test.

As we mentioned previously, monoamine changes in vivo involve a complicated process. Accordingly, in the present study, we assessed 071031B's effect on monoamine levels and focused on the changes under depressive state. Although reserpine was an antihypertensive drug, clinical research in 1970's found that reserpine treatment would lead to depression by depleting monoamine levels. Indeed, our results in HPLC analyses revealed that pretreatment with reserpine at 5 mg/kg induced significant decreases in three monoamine transmitters, i.e., 5-HT, NE, DA, and increases in values of 5-HIAA/5-HT and DOPAC/DA in various brain regions. 071031B or duloxetine reduced 5-HT turnover in all three regions of reserpinized rats, while their increasing effects on 5-HT or NE levels were brain-region specific. Only in hypothalamus did 071031B or duloxetine increase 5-HT and NE levels simultaneously, whereas no effects were observed in hippocampus. In prefrontal cortex, only one type of transmitters was increased by duloxetine at 20 mg/kg or 071031B at 10 and 20 mg/kg. However, although there was no statistical significance, the other type of transmitters was also increased by more than 50% following duloxetine or 071031B treatment. Frontal cortex is of particular interest for SNRIs research because of its extensive serotonergic and noradrenergic innervations and interactions (13). Actually, we found that the potency of 071031B to NE uptake inhibition was greater in frontal cortex than hippocampus (data not shown). Gehlert et al (14) found similar brain region difference, and they reported that the potency to inhibit NE uptake by duloxetine was greater in hypothalamus. Like frontal cortex, hypothalamus also received extensive serotonergic and noradrenergic innervations. The mechanisms of this brain-specific response were not clear, yet may be related to two reasons. First, density of serotonergic and noradrenergic nerve fiber varies between brain areas, and interactions between 5-HT and NE differ too. Second, different drugs may have different characteristics, including pharmacodynamic and pharmacokinetic profiles. Muneoka et al (15) also demonstrated that SNRIs induced changes in monoamines were brain-region specific. Both 071031B and duloxetine showed no effect on DA or its turn over in striatum.

Chronic unpredictable stress (CUS) model is the most commonly used animal model in researching depression, for it induces similar behavioral deficits and neurochemical disturbances as depressive patients (16). The antidepressant effects of 071031B in CUS model were described previously (5), and using same animals, our current study demonstrated that 071031B was effective on CUS induced NE depletion. HPLC analyses revealed that a four-week stress regime presented in Table 1 induced significant reduction in NE level in prefrontal cortex, without influencing 5-HT level and its turnover in prefrontal cortex or DA and its turnover in striatum. Rats treated with 071031B or duloxetine produced significant increases in NE levels in prefrontal cortex. Interestingly, it is worth noting that fluoxetine, as a selective serotonin reuptake inhibitor, restored NE level in prefrontal cortex in CUS rats to the same extent as duloxetine or 071031B. In fact, Bymaster et al. (17) have shown that fluoxetine, but not other SSRIs, increases NE and DA extracellular levels in prefrontal cortex without effects on blocking NET at the same dose, which is proposed to be due to fluoxetine's blocking effects on 5-HT_{2C} receptors. To our knowledge, our study reported the increasing effects of fluoxetine on NE levels in CUS model for the first time. Numerous studies reported relatively consistent behavioral endpoints in CUS model, while neurochemical endpoints varied between (or indeed, within) laboratories. For instance, Harro et al found that 2w variable stress only induced HVA increasing in prefrontal cortex, without affecting 5-HT and NE levels in prefrontal cortex or hippocampus (18), whereas Bhutani et al reported that 3w chronic stress decreased 5-HT and DA levels in whole brain, without effects on NE or 5-HIAA (19). This inconsistence may be associated with variable experimental procedures, e.g., different stressors, sequence, intensity, duration, and so on. In fact, depression patients are proposed to show two types of characteristics, 'noradrenergic' depression or 'serotonergic' depression. Under our experimental condition, changes in NE system, but not 5-HT or DA, were involved in neurochemical endpoints in CUS model and antidepressant effects of 071031B. However, we must bear in mind that, in this study, we did not determine monoamine levels in hippocampus or other areas; therefore, we can not rule out the possibility of a brain-region specific effect.

5. Conclusion

Our study demonstrates that 071031B produces potent inhibition of 5-HT and NE uptake in vivo. Moreover, this study supports and extends our previous studies, and suggests that 071031B may be an effective antidepressant requiring clinical evaluation.

Conflicts of interest

The authors declare no conflict of interest.

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